

## **Supplementary figures**

**Supplementary Figure 1.** Sequence alignment of IDH2 reveals evolutionarily invariant lysine residues. Lysines 256, 263, and 413 (shaded in gray) are invariant among diverse species. Sequence alignment was performed using The Uniprot Consortium sequence alignment tool (The UniProt Consortium, Ongoing and future developments at the Universal Protein Resource, Nucleic Acids Res. 39: D214-D219 (2011).) Asterisks (\*) indicate invariant amino acids, : indicate conserved amino acids.

**Supplementary Figure 2.** A. Western blotting confirms stable expression of SIRT3. Mitochondrial extracts from four stable cell pools were made by anti-FLAG immunoprecipitation. Anti-FLAG western blotting confirmed stable expression.

B. Western blotting confirms WT and mutant IDH2 stable expression in *Sirt3*<sup>-/-</sup> MEFs. Mitochondrial extracts from four stable cell pools were prepared and anti-FLAG western blotting confirmed stable expression.

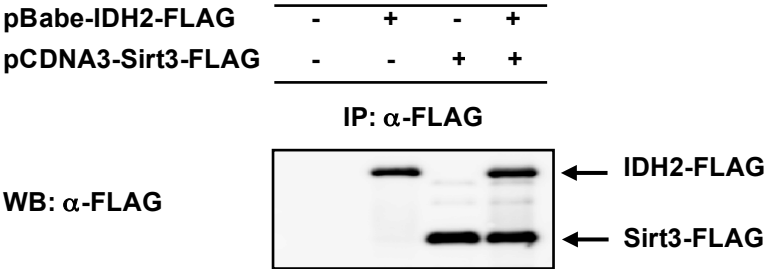
**Supplementary Figure 3.** Structural analysis of IDH2 shows highly charged NADP<sup>+</sup> binding pocket. *A.* Structure of porcine mitochondrial IDH2 (PDB: 1LWD) with NADP<sup>+</sup> modeled into binding pocket. Lysine 413 is indicated on one subunit of the homodimer is indicated. *B.* Electrostatic potential maps of WT IDH2 or IDH2 with lysine mutated to glutamine. Black (blue) represents positively charged regions, white (red) represents negatively charged regions. The change of lysine to glutamine dramatically changes the surface charge in the NADP<sup>+</sup> binding pocket that may be needed for the proper binding conformation of NADP<sup>+</sup>. All images generated with PyMol.

**Supplementary Table 1.** Summary of kinetic values obtained via activity assays with the varying IDH2 proteins indicated.

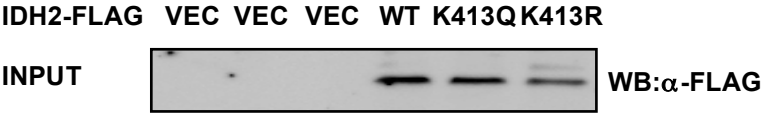
Yu et.al Supplementary Figure 1

	250	260	270	410
Homo sapiens	PLYMSTKNTILKAYDGRFKDIFQEIFDK			ETVES-GAMTKDLAG
Mus musculus	PLYLSTKNTILKAYDGRFKDIFQEIFDK			QTVES-GAMTKDLAG
Saccharomyces cerevisiae	PLYMSTKNTILKAYDGRFKDIFQDIFEK			ETVES-GIMTKDLAG
Xenopus laevis	PLYMSTKNTILKAYDGRFKDIFQDIFEK			ETVES-GVMTKDLAG
Danio rerio	PLYLSTKNTILKKYDGRFKDIFQDIYEK			DTVEE-GKMTKDLSI
Caenorhabditis elegans	NLFLSTKNTILKKYDGRFKDIFQEVYEA			NTVQQDGIMTKDLAL
Arabidopsis thaliana	PLYLSTKNTILKKYDGRFKDIFQEVYEA			NTVET-GKMTKDLSI
	*::*****	*****:::		::*::

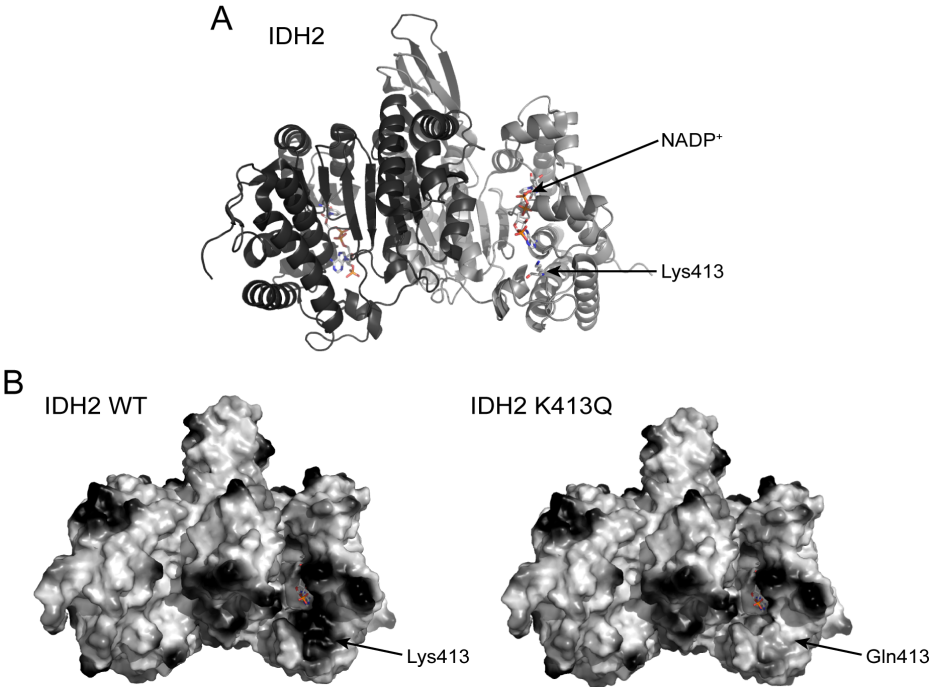
**A**



**B**



Yu et.al Supplementary Figure 3



**Steady-state kinetic analysis of WT and mutants of human IDH2**

		<b>NADP<sup>+</sup></b>	<b>Isocitrate</b>
<b>WT</b>	$K_m$ ( $\mu\text{M}$ )	$3.1 \pm 0.6$	$6.0 \pm 0.8$
	$V_{\max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	$42.1 \pm 2.5$	$43.4 \pm 2.4$
<b>K413Ac</b>	$K_m$ ( $\mu\text{M}$ )	$9.8 \pm 3.3$	$6.1 \pm 1.6$
	$V_{\max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	$3.0 \pm 0.3$	$2.6 \pm 0.2$
<b>K413R</b>	$K_m$ ( $\mu\text{M}$ )	$4.2 \pm 1.0$	$6.6 \pm 0.4$
	$V_{\max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	$31.2 \pm 2.6$	$39.2 \pm 1.1$
<b>K413Q</b>	$K_m$ ( $\mu\text{M}$ )	$15.1 \pm 3.2$	$9.6 \pm 3.7$
	$V_{\max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	$2.3 \pm 0.3$	$1.8 \pm 0.3$